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Foliar Nickel Application Alleviates Detrimental Effects of Glyphosate Drift on Yield and Seed Quality of Wheat

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Supporting Information

ABSTRACT: Glyphosate drift to nontarget crops causes growth aberrations and yield losses. This herbicide can also interact with divalent nutrients and form poorly soluble complexes. The possibility of using nickel (Ni), an essential divalent metal, for alleviating glyphosate drift damage to wheat was investigated in this study. Effects of Ni applications on various growth parameters, seed yield, and quality of durum wheat (*Triticum durum*) treated with sublethal glyphosate at different developmental stages were investigated in greenhouse experiments. Nickel concentrations of various plant parts and glyphosate-induced shikimate accumulation were measured. Foliar but not soil Ni applications significantly reduced glyphosate injuries including yield losses, stunting, and excessive tillering. Both shoot and grain Ni concentrations were enhanced by foliar Ni treatment. Seed germination and seedling vigor were impaired by glyphosate and improved by foliar Ni application to parental plants. Foliar Ni application appears to have a great potential to ameliorate glyphosate drift injury to wheat.

KEYWORDS: *glyphosate, nickel, durum wheat (Triticum durum), drift, grain yield, seed quality*

INTRODUCTION

Glyphosate (*N*-[phosphonomethyl]glycine) is a systemic and nonselective postemergence herbicide. Due to its high effectiveness and low cost as well as the widespread adoption of glyphosate-resistant (GR) transgenic crops and no-tillage cropping system, glyphosate has become the most commonly applied herbicide worldwide.[1,2] Glyphosate exerts its main herbicidal activity by specifically inhibiting 5-enolpyruvylshikimate 3-phoshate synthase (EPSPS) in the shikimate pathway and thus blocking the biosynthesis of aromatic amino acids, auxin, lignin, and other phenolic compounds.[2,3] The inhibition of this critical pathway leads to impairments in protein and photosynthetic carbon metabolism.[1,4] Foliar-applied glyphosate moves to actively growing shoot and root tips with high sink activities and causes shikimate accumulation in these young tissues.[5−7]

Glyphosate drift to nontarget crops is a growing practical concern. Herbicide drift rates to susceptible plants may be as high as 10% of the recommended application rates.[8] In numerous economically important nonresistant crops, including soybean (*Glycine max*),[7−9] sunflower (*Helianthus annuus*),[10] potato (*Solanum tuberosum*),[11] wheat (*Triticum aestivum*),[12−14] sorghum (*Sorghum bicolor*),[12] rice (*Oryza sativa*), and corn (*Zea mays*),[15,16] glyphosate drift simulation studies demonstrated significant growth aberrations and yield reductions. In wheat, 10% of the labeled usage rate of glyphosate was shown to be able to cause yield losses by >90%, depending on climatic conditions and treatment stage.[13] Another study documented yield losses by up to 70% when wheat was sprayed with practically relevant drift doses of glyphosate at the first node stage.[14]

Besides yield loss, the typical growth anomalies associated with sublethal glyphosate injury in grasses are reduced stem elongation (stunting) and increased tillering.[12,14] Dicots also exhibit abnormal growth symptoms in response to glyphosate drift, including increased axillary branching[17,18] and epinasty.[1,17] Reportedly, the disruption of phytohormone balance by glyphosate contributes to sublethal glyphosate injury.[1] Induction of tillering in grasses and axillary branching in dicots by glyphosate indicates a temporary loss of apical dominance, which was associated with glyphosate-induced inhibition of polar auxin transport from the actively growing apex.[1,17,18] According to Baur,[17] glyphosate may do so indirectly by inducing the production of ethylene, known to disrupt auxin transport.[19,20] Glyphosate-induced ethylene production was documented for common bean (*Phaseolus vulgaris*)[21] and white birch (*Betula papyrifera*).[22]

Another aspect of glyphosate drift to nontarget plants is the effects of glyphosate on mineral nutrition. Reduced tissue concentrations as well as impaired uptake and translocation of essential cationic nutrients, particularly Ca, Mg, Fe, and Mn, were documented in various species such as soybean,[7,23,24] sunflower,[6,10] and turfgrass.[25] As a chelating agent, glyphosate can form complexes of various stabilities with divalent metal cations.[26,27] Due to such interaction, the presence of several divalent cations including Ca, Mg, Mn, and Zn in spray solutions was shown to lower the herbicidal effect of glyphosate.[28−30] The formation of poorly soluble glyphosate−metal complexes may also be relevant in planta or in the rhizosphere, reducing the root uptake and translocation of these nutrients within plant tissues.[7,10] In a recent paper, it was suggested that impairment of root growth by glyphosate may also contribute to reduced mineral uptake in nontarget plants.[27]

As a less-studied divalent cation, nickel (Ni) was also recently reported to be affected by glyphosate.[31] In that study, impaired

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nodulation observed in glyphosate-treated glyphosate-resistant soybean plants was explained by glyphosate-induced reduction in Ni availability to nitrogen-fixing symbiotic bacteria. Nickel is known to be an essential micronutrient for higher plants, although the knowledge of its functions as a plant nutrient is still rather limited.[32,33] In plants, urease is still the only enzyme known to require a Ni cofactor, responsible for the assimilation of urea generated endogenously or absorbed from the environment.[33] It is well established that sufficient Ni is critical for the utilization of urea fertilizers and the prevention of urea accumulation to toxic levels.[34,35] Nickel deficiency, which was clearly demonstrated under field conditions as the cause of mouse-ear symptoms in pecan nut (*Carya illinoinensis*),[36] can disrupt the amino acid and organic acid homeostasis in crops.[37] Nickel ions are also effective inhibitors of ethylene biosynthesis in plants.[33,38,39] Possibly, Ni depresses aminocyclopropane carboxylate (ACC) oxidase, which catalyzes the conversion of ACC to ethylene, by replacing the Fe cofactor.[40,41] More recently, Zheng et al.[42] showed that preharvest application of Ni as an ethylene inhibitor could significantly prolong the shelf life of Japanese persimmon fruits (*Diospyros kaki*).

Due to the relatively high in vitro affinity of Ni^{2+} to glyphosate[26] and its role as an ethylene biosynthesis inhibitor in planta, Ni may interact with glyphosate in crops directly at a chemical and/or indirectly at a functional level. To our knowledge, this is the first report on the effects of Ni on glyphosate drift injury in a glyphosate-susceptible crop. This study was conducted to investigate the possibility of using soil and foliar Ni applications for alleviating glyphosate drift damage to durum wheat (*Triticum durum*). Glyphosate drift was simulated under greenhouse conditions by applying different levels of sublethal glyphosate to wheat plants at different developmental stages. Visual injury symptoms, various growth parameters, shikimic acid accumulation, grain yield, and seed germination were investigated to demonstrate the effects of Ni on glyphosate damage.

MATERIALS AND METHODS

Plant Growth Conditions. In all experiments, durum wheat (*T. durum* cv. Balcali2000) plants were grown under natural daylight in a computer-controlled greenhouse. With the help of a heating and evaporative cooling system, the daytime temperature was kept at 25 ± 4 °C and the night time temperature at 20 ± 4 °C in the greenhouse located at the following geographic coordinates: 40° 53′ 25″ N, 29° 22′ 47″ E.

The soil used in all soil experiments was a calcareous (18% $CaCO_3$) inceptisol from a semiarid region of Central Anatolia. This clayey-loam soil had a high pH (8.0 in dH_2O) and low organic matter content (1.5%). The diethylenetriaminepentaacetic acid (DTPA)-extractable micronutrient concentrations of the soil were as follows: 1.22 mg/kg Ni, 0.13 mg/kg Zn, 2.73 mg/kg Fe, 2.45 mg/kg Mn, and 0.75 mg/kg Cu.

Each pot was filled with 3 kg of air-dry soil. Before sowing, the following mineral nutrients were added to each pot in the form of concentrated solutions and mixed with the soil thoroughly (per kg dry soil): 300 mg of N as $Ca(NO_3)_2{\cdot}4H_2O$, 100 mg P as KH_2PO_4, and 25 mg S as K_2SO_4. Because the experimental soil was Zn-deficient, pots received 5 mg Zn/kg soil as $ZnSO_4{\cdot}7H_2O$. For soil Ni application, Ni was added with other nutrients in the form of $NiCl_2{\cdot}6H_2O$. The plants that were grown until grain maturation were fertilized with an additional 100 mg N/kg soil at anthesis. All soil experiments had completely randomized designs, and each treatment group consisted of four pot replicates with each containing six individual plants. Plants were watered with deionized water (dH_2O) regularly throughout the experiment.

Experimental Design and Procedure. The recommended application dose of Roundup STAR (Monsanto) containing 441 g/L *N*-(phosphonomethyl)glycine (glyphosate) potassium salt is 300 mL/1000 m^2 in 30 L of water. To mimic glyphosate drift in the first experiment, 1 and 1.5% of the recommended dose were selected, corresponding to 0.21 and 0.32 mM glyphosate, respectively. (Throughout the paper, *n*% glyphosate refers to *n*% of the recommended herbicidal glyphosate application dose.) There were five Ni treatment groups in this experiment: One group was left untreated as control, another one was fertilized with 2 mg Ni/kg soil at the beginning of the experiment, and the remaining three groups were sprayed with Ni at different concentrations when the plants were 33 days old and at tillering stage (Zadoks Stage (ZS): 21−24). Nickel sprays contained 0.002% (referred to as low), 0.01% (referred to as medium), or 0.02% (referred to as high) (w/v) $NiCl_2{\cdot}6H_2O$ (corresponding to 0.08, 0.42, and 0.84 mM Ni, respectively) and 0.01% (w/v) Tween-20 as surfactant. The remaining pots were sprayed with the same amount of dH_2O containing only 0.01% (w/v) Tween-20. Two days later, one-third of the pots were treated with 1% and one-third with 1.5% glyphosate, whereas the remaining one-third were sprayed with just water as control. When control plants were at booting stage (ZS: 45−47), 50 days after sowing (DAS), main stem heights (up to the joint of the youngest leaf blade) were measured. Samples taken from the second youngest fully expanded leaves, which were the youngest common non-necrotic leaves in all glyphosate treatment groups, were used for shikimate analysis according to the method described by Ozturk et al.[6] Plant shoots were harvested, washed three times with dH_2O, and dried at 70 °C for 2 days. The dry samples were ground, digested, and analyzed for Ni concentration as described below.

The second soil experiment was designed as a fully factorial experiment where wheat plants were grown until grain maturation. At the beginning, half of the plants were fertilized with 2 mg Ni/kg soil. When the main stems were swelling (ZS: 41−45), 43 days after sowing, half of the pots were sprayed with 0.01% (w/v) $NiCl_2{\cdot}6H_2O$ and 0.01% (w/v) Tween-20 and the rest with only 0.01% (w/v) Tween-20. Two days later, half of the pots were treated with 1% (of rec) glyphosate, whereas the remaining were sprayed with just water. When the plants completely senesced, the straw and spikes were harvested separately. The samples were dried, ground, digested, and analyzed for Ni as described below.

The effects of the timing of glyphosate treatment were studied in the third soil experiment. Plants were treated with glyphosate either at tillering (ZS: 21−24; 29 days after sowing) or at booting (ZS: 47−49; 50 days after sowing) stage. Foliar Ni applications were carried out two days before glyphosate treatments. For foliar Ni application, plants were sprayed with 0.01% (w/v) $NiCl_2{\cdot}6H_2O$ solution containing 0.01% (w/v) Tween-20 or only Tween-20. Glyphosate treatments were conducted by spraying plants with 0.5 or 1% of the recommended dose or just dH_2O. Half of the earlier treated pots were harvested when control plants reached were at the stem elongation (ZS: 37−39; 43 days after sowing), whereas the other half as well as the later treated pots were grown until grain maturation. The main stem heights (up to the joint of the youngest leaf blade for vegetative stage plants and up to the beginning of the spike for mature plants) were measured just before harvest in all treatment groups. Whole shoots of vegetative stage plants were harvested and washed with dH_2O. In the case of mature plants, the straw and spikes were harvested separately. All samples were dried at 70 °C for 2 days. The dry samples were ground, digested, and analyzed for Ni concentration as described below.

A germination test was conducted using the seeds produced by the plants subjected to glyphosate at booting in the third soil experiment. From each seed batch (produced by plants grown in one pot), 50 seeds were selected randomly. Because the soil experiment had four replicates, the germination test was also a four-replicate experiment wherein each glyphosate × Ni group was represented by 200 seeds in total. Seeds were sown in perlite moistened with 2 mM $CaSO_4{\cdot}2H_2O$ and germinated in the greenhouse for 8 days. The germination

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percentage and shoot (coleoptile + primary leaf) length of the seedlings were determined.

Digestion and Mineral Analysis. Dried and ground samples (0.3−0.4 g) were acid digested by using 2 mL of 30% H_2O_2 and 5 mL of 65% HNO_3 per sample in a closed-vessel microwave system (MarsExpress; CEM Corp., Matthews, NC, USA). Inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd., Mulgrave, Australia) was used to analyze the digested samples for their mineral element (Ni, Ca, Mg, Zn, and Fe) concentrations.

Statistical Analysis. All reported values are means of four pot replicates. The JMP software was used for statistical analysis. The significance of the effects of the Ni and glyphosate treatments and their interaction on the reported traits was evaluated by analysis of variance (ANOVA). Significant differences between means were determined using Tukey's protected honestly significant difference (HSD) test at 5% significance.

RESULTS

In the first experiment, wherein the impacts of soil and foliar applications of Ni on actively growing wheat plants subject to sublethal glyphosate concentrations were studied, the shoot dry weight and main stem height were significantly affected by Ni and glyphosate treatments as well as their interaction (Table 1A,B). When the plants were harvested 15 days after glyphosate treatment, 1 and 1.5% of the recommended lethal glyphosate dose reduced the shoot biomass on average by 30 and 40%, respectively (Figure 1; Table 1A). Low foliar and soil Ni applications had no significant effect on the shoot dry weight, whereas medium and high rates of foliar Ni reduced the loss in shoot biomass of wheat plants treated with 1% glyphosate. The main stem height, which was halved by 1% glyphosate in the absence of any Ni application, was almost completely restored to control levels by medium and high foliar Ni doses (Figure 1; Table 1B). In the case of 1.5% glyphosate, the main stem height was even more drastically reduced, and none of the Ni treatments provided any benefit.

At the time of harvest, the shikimate concentration measurements revealed no shikimate accumulation in the young leaves of wheat plants treated with 1% glyphosate (Table 1C), probably because the time between glyphosate application and leaf sampling was too long (14 days), and the analysis could not be carried out in the youngest leaves and shoot tips, which died upon 1.5% glyphosate treatment (see Discussion). However, the application of 1.5% glyphosate caused a statistically significant 30% increase in shikimate levels, which was not prevented by any Ni treatment. Glyphosate and soil Ni treatments did not affect the shoot Ni concentration, whereas increasing levels of foliar Ni application resulted in marked increases in shoot Ni concentration (Table 1D).

The effects of soil and foliar Ni applications on glyphosate-induced grain yield loss in wheat were investigated in the next experiment. On the basis of the results of the previous experiment, 1% glyphosate and the medium foliar Ni rate (0.01% $NiCl_2{\cdot}6H_20$) were selected as effective application levels for this study. Dramatic yield losses were observed in the absence of foliar Ni treatment when the plants were sprayed with glyphosate at booting (Table 2). Irrespective of soil Ni application, foliar Ni treatment quadrupled the grain yield of wheat plants subjected to glyphosate by preventing nearly 75% of the damage caused by glyphosate. Soil application of Ni did not have any significant effect on grain yield under these experimental conditions. In contrast to grain yield, straw dry weight did not exhibit marked responses to glyphosate or Ni

Table 1. Effects of Low (0.002% $NiCl_2{\cdot}6H_2O$), Medium (0.01% $NiCl_2{\cdot}6H_2O$), and High (0.02% $NiCl_2{\cdot}6H_2O$) Foliar Ni (33 DAS), Soil (2 mg/kg) Ni, and Glyphosate (35 DAS) Applications on Shoot Dry Weight (A), Main Stem Height (B), Shikimate Concentration (C), and Shoot Ni Concentration (D) of 50-Day-Old Durum Wheat (*Triticum durum* cv. Balcali2000) Plants Grown under Greenhouse Conditions

(A) Shoot DW (g/Plant)			
	glyphosate dose (% of rec)		
Ni application	0	1.0	1.5
no Ni	2.26 ± 0.08	1.45 ± 0.18	1.21 ± 0.09
low foliar Ni	2.07 ± 0.14	1.28 ± 0.07	1.19 ± 0.18
medium foliar Ni	2.10 ± 0.10	1.64 ± 0.04	1.13 ± 0.10
high foliar Ni	2.16 ± 0.08	1.82 ± 0.17	1.24 ± 0.13
soil Ni	2.21 ± 0.09	1.51 ± 0.20	1.45 ± 0.11
$HSD_{0.05}$ (Ni; Gly; NixGly) = (0.15; 0.10; 0.32)			
(B)Main Stem Height (cm)			
	glyphosate dose (% of rec)		
Ni application	0	1.0	1.5
no Ni	35.1 ± 1.6	17.0 ± 1.3	13.1 ± 1.0
low foliar Ni	33.4 ± 2.1	16.9 ± 3.3	13.6 ± 1.1
medium foliar Ni	33.6 ± 2.6	30.1 ± 4.1	13.5 ± 1.1
high foliar Ni	34.5 ± 2.6	29.4 ± 2.9	14.1 ± 1.6
soil Ni	34.5 ± 2.5	20.1 ± 3.2	12.8 ± 1.0
$HSD_{0.05}$ (Ni; Gly; NixGly) = (2.4; 1.6; 5.3)			
(C) Shikimate Concentration (μmol/g FW)			
	glyphosate dose (% of rec)		
Ni application	0	1.0	1.5
no Ni	1.69 ± 0.07	1.64 ± 0.32	2.14 ± 0.49
low foliar Ni	1.43 ± 0.22	1.48 ± 0.23	2.00 ± 0.20
medium foliar Ni	1.47 ± 0.30	1.39 ± 0.06	1.89 ± 0.42
high foliar Ni	1.57 ± 0.14	1.47 ± 0.52	2.13 ± 0.46
soil Ni	1.50 ± 0.48	1.55 ± 0.08	1.99 ± 0.27
$HSD_{0.05}$ (Ni; Gly; NixGly) = (ns; 0.25; ns)			
(D) Shoot Ni Concentration (mg/kg)			
	glyphosate dose (% of rec)		
Ni application	0	1.0	1.5
no Ni	2.9 ± 0.4	2.1 ± 0.3	1.7 ± 0.1
low foliar Ni	3.3 ± 0.4	3.8 ± 0.2	4.0 ± 0.5
medium foliar Ni	9.4 ± 1.1	11.5 ± 1.3	10.1 ± 0.6
high foliar Ni	19.3 ± 2.0	20.6 ± 2.9	24.8 ± 3.6
soil Ni	2.8 ± 0.3	2.6 ± 0.1	2.1 ± 0.4
$HSD_{0.05}$ (Ni; Gly; NixGly) = (1.6; ns; 3.5)			

applications. Glyphosate and soil Ni treatments tended to slightly decrease straw dry weight, whereas foliar Ni did not affect straw dry weight at all. Grain Ni concentration increased markedly in response to foliar Ni treatment, but it did not respond to soil Ni application. In the absence of foliar Ni, glyphosate-treated plants produced grains with higher Ni concentrations. Straw Ni concentration showed a 4-fold increase upon foliar Ni application, irrespective of glyphosate and soil Ni treatments.

The next experiment was conducted to study the interactive effects of foliar Ni and sublethal glyphosate at different application stages on vegetative growth and grain yield of wheat. When foliar treatments were carried out at tillering and plants were harvested 2 weeks later at stem elongation, dwarfing and excessive tillering were observed in plants treated

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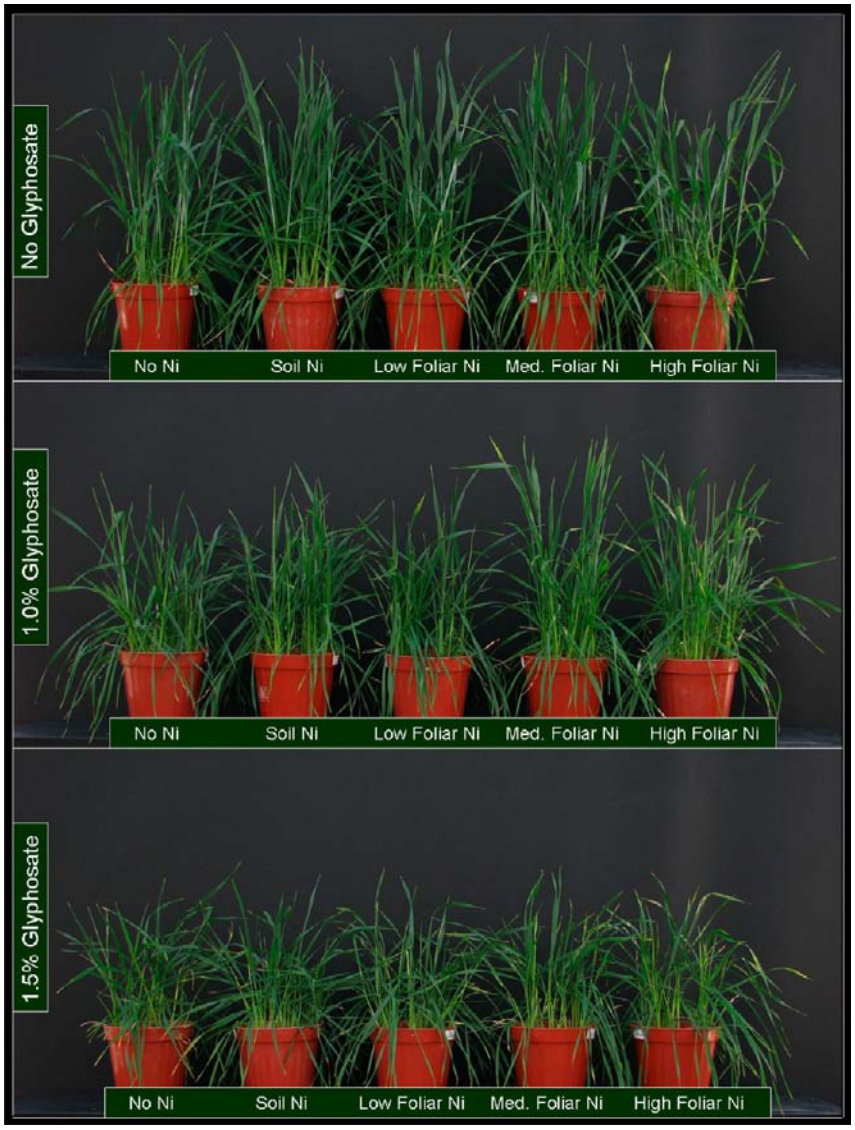

Figure 1. Effects of low (0.002% $NiCl_2 \cdot 6H_2O$), medium (0.01% $NiCl_2 \cdot 6H_2O$), and high (0.02% $NiCl_2 \cdot 6H_2O$) foliar Ni (33 DAS), soil (2 mg/kg) Ni, and glyphosate (35 DAS) applications on 50-day-old durum wheat (*Triticum durum* cv. Balcali2000) plants grown under greenhouse conditions.

Table 2. Effects of Soil Ni, Foliar Ni (43 DAS), and Glyphosate (45 DAS) Treatments on Grain Yield, Straw Dry Weight, and Grain Ni and Straw Ni Concentrations of Durum Wheat (*Triticum durum* cv. Balcali2000) Plants Grown under Greenhouse Conditions

soil Ni (A) (mg/kg)	foliar Ni (B) (% $NiCl_2 \cdot 6H_2O$)	glyphosate dose (C) (% of std)	grain yield[a] (g/plant)	straw DW[b] (g/plant)	grain Ni concn[c] (mg/kg)	straw Ni concn[d] (mg/kg)
0	0	0	3.5 ± 0.1	2.4 ± 0.1	2.6 ± 0.4	0.8 ± 0.1
		1.0	0.5 ± 0.3	2.4 ± 0.2	5.8 ± 1.7	0.9 ± 0.1
	0.01	0	3.2 ± 0.3	2.4 ± 0.2	8.3 ± 1.8	5.2 ± 1.2
		1.0	2.4 ± 0.2	2.1 ± 0.1	6.8 ± 1.3	5.1 ± 0.9
2	0	0	3.2 ± 0.1	2.1 ± 0.1	2.9 ± 0.3	1.0 ± 0.1
		1.0	0.7 ± 0.1	1.9 ± 0.1	4.9 ± 0.4	1.1 ± 0.3
	0.01	0	3.1 ± 0.2	2.2 ± 0.1	8.2 ± 1.6	5.3 ± 0.1
		1.0	2.4 ± 0.3	2.2 ± 0.1	8.9 ± 1.4	6.6 ± 0.8

[a]Grain yield: $HSD_{0.05}$ (A; B; C; AxB; AxC; BxC; AxBxC) = (ns; 0.2; 0.2; 0.3; ns; 0.3; ns). [b]Straw dry weight: $HSD_{0.05}$ (A; B; C; AxB; AxC; BxC; AxBxC) = (0.1; ns; 0.1; 0.2; ns; ns; ns). [c]Grain Ni concentration: $HSD_{0.05}$ (A; B; C; AxB; AxC; BxC; AxBxC) = (ns; 0.9; 0.9; ns; ns; 1.7; ns). [d]Straw Ni concentration: HSD0.05 (A; B; C; AxB; AxC; BxC; AxBxC) = (ns; 0.5; ns; ns; ns; ns; ns).

with 1% glyphosate but not sprayed with Ni (Figure 2). Neither shoot dry weight nor main stem height was significantly affected by 0.5% glyphosate (Supporting Table 1 in the Supporting Information). In the absence of Ni, 1% glyphosate

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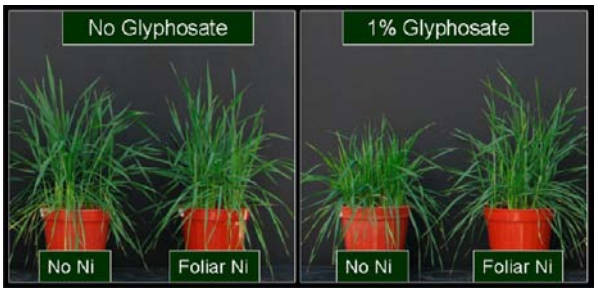

Figure 2. Effects of foliar Ni (0.01% $NiCl_2 \cdot 6H_2O$; 27 DAS) and glyphosate (1% of rec; 29 DAS) treatments on 43-day-old durum wheat (*T. durum* cv. Balcali2000) plants grown under greenhouse conditions.

decreased shoot dry weight by 25%. This reduction of shoot biomass by glyphosate was significantly but only partially (by 40%) prevented by foliar Ni. In agreement with the results of the first experiment, an even more pronounced protective effect of foliar Ni was observed in the context of main stem height (Figures 1 and 2; Table 1; Supporting Table 1). When the plants were not sprayed with Ni, 1% glyphosate reduced main stem height by 40% (Figure 2; Supporting Table 1). Foliar Ni application completely protected the plants from the effects of glyphosate on stem elongation. Shoot Ni concentrations of Ni-sprayed plants were on average 70% higher than those of nontreated plants (Supporting Table 1).

Application of 0.5% glyphosate to wheat plants at tillering did not cause a considerable yield loss, whereas the same glyphosate dose reduced grain yield significantly when applied at booting (Table 3). The yield loss due to 0.5% glyphosate at booting was about 20% in plants not treated with foliar Ni, but only 10% in Ni-sprayed plants. However, 1% glyphosate reduced grain yield when applied not only at booting but also at tillering. At booting, it halved the grain yield in the absence of foliar Ni, but the loss was limited to 20% in the presence of foliar Ni. The protective effect of foliar Ni was also observed at tillering, where foliar Ni totally prevented a yield loss of 25% due to 1% glyphosate. The number of grains produced per plant exhibited trends similar to grain yield in response to glyphosate and Ni treatments. At booting, foliar Ni effectively counteracted glyphosate, which caused marked grain number reductions in a dose-dependent manner. To a lesser extent, the negative effect of glyphosate on grain number was also observed when 1% glyphosate was applied at tillering, and it was almost completely eliminated by foliar Ni. Foliar Ni application enhanced grain Ni concentrations significantly at both treatment stages, but particularly when applied at booting.

In contrast to yield and grain number, straw dry weight was unaffected by glyphosate, foliar Ni, or their interaction (Table 3). Nevertheless, stem elongation was impaired by glyphosate. The final main stem height was significantly lowered by only 1% glyphosate in the case of tillering application, but by both 0.5 and 1% glyphosate in the case of booting application (Figure 3; Table 3). Foliar Ni treatment was partially or completely successful in preventing plants from the dwarfing effect of glyphosate. The plants treated with 1% glyphosate at tillering but not sprayed with Ni were not only dwarfed, but also bore greater numbers of tillers during the generative development (Figure 3), as was also the case during the vegetative stage (Figure 2).

Plants subjected to glyphosate at booting produced deformed grains (Figure 4A). Both the number of wrinkled seeds and the severity of deformation increased with increasing glyphosate concentration. This form disorder was not observed in seeds produced by Ni-sprayed plants. To investigate if this visual phenomenon was also linked to a physiological impairment, these seeds were germinated (Figure 4B). The germination test revealed that germination was adversely affected by glyphosate and significantly improved by foliar Ni application to parental plants (Figure 4B; Table 4). In addition to lower germination percentages, seeds of 1% glyphosate-treated plants exhibited impaired shoot growth. In the absence of foliar Ni application to parental plants, the mean shoot length of these seedlings was 30% lower than that of controls (Table 4). Foliar Ni treatment

Table 3. Grain Yield, Grain Number, Straw Dry Weight, Main Stem Height, and Grain Ni Concentration of Durum Wheat (*Triticum durum* cv. Balcali2000) Plants Treated with Foliar Ni and Glyphosate at Tillering or Booting under Greenhouse Conditions

treatment stage	foliar Ni (% $NiCl_2 \cdot 6H_2O$)	glyphosate dose (% of rec)	grain tield (g/plant)	grain no. (per plant)	grain Ni concn (mg/kg)	straw DW (g/plant)	main stem height (cm)
tillering[a]	0	0	4.8 ± 0.3	111 ± 8	2.5 ± 0.3	3.8 ± 0.1	73 ± 4
		0.5	4.6 ± 0.3	108 ± 5	2.3 ± 0.2	3.6 ± 0.3	71 ± 3
		1.0	3.5 ± 0.2	90 ± 18	2.6 ± 0.5	3.8 ± 0.2	62 ± 2
	0.01	0	4.7 ± 0.2	117 ± 6	2.8 ± 0.2	4.0 ± 0.2	72 ± 3
		0.5	4.5 ± 0.2	112 ± 11	2.6 ± 0.4	3.8 ± 0.2	73 ± 2
		1.0	4.3 ± 0.2	108 ± 8	2.9 ± 0.3	3.9 ± 0.1	71 ± 3
booting[b]	0	0	4.7 ± 0.3	110 ± 7	2.9 ± 0.7	3.9 ± 0.1	73 ± 3
		0.5	3.8 ± 0.5	75 ± 16	2.9 ± 0.4	3.9 ± 0.4	66 ± 3
		1.0	2.3 ± 0.4	51 ± 11	4.0 ± 0.6	4.1 ± 0.4	54 ± 5
	0.01	0	4.6 ± 0.4	106 ± 15	5.1 ± 0.7	3.9 ± 0.4	71 ± 2
		0.5	4.1 ± 0.3	109 ± 6	5.1 ± 0.6	4.2 ± 0.2	69 ± 1
		1.0	3.7 ± 0.7	81 ± 19	4.8 ± 0.8	4.1 ± 0.5	63 ± 5

[a]Tillering: grain yield, $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (0.4; 0.2; 0.7); grain no., $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (15; 10; ns); grain Ni concn, $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (ns; 0.3; ns); straw DW, $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (ns; ns; ns); main stem height, $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (4; 2; 7). [b]Booting: grain yield, $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (0.6; 0.4; 1.1); grain no., $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (17; 12; 29); grain Ni concn, $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (ns; 0.6; ns); straw DW, $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (ns; ns; ns); main stem height, $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (4; 2; 6).

Figure 3. Durum wheat (*T. durum* cv. Balcali2000) plants treated with foliar Ni (0.01% $NiCl_2 \cdot 6H_2O$) and glyphosate (1% of rec) at tillering or booting under greenhouse conditions.

Figure 4. (A) Seeds produced by durum wheat (*T. durum* cv. Balcali2000) plants treated with foliar Ni (0.01% $NiCl_2 \cdot 6H_2O$) and glyphosate (0.5 or 1% of rec) at booting. (B) 8-Day-old durum wheat seedlings grown in perlite from seeds shown in (A).

of the previous generation almost fully prevented this growth disorder.

DISCUSSION

Reduced vegetative biomass production in young plants subjected to sublethal glyphosate rates was documented in the literature for several crops[7,10] and can be explained by impairments of various physiological processes including photosynthetic carbon fixation.[4,31] Conformably, young wheat plants treated with drift doses of glyphosate in this study produced lower biomass than control plants (Figures 1 and 2; Table 1A). Also in agreement with previous glyphosate drift simulation studies on cereals,[14,15] the stem elongation of wheat plants was inhibited by sublethal concentrations of glyphosate (Figures 1−3; Tables 1B and 3; Supporting Table 1). Foliar applications of Ni at sufficiently high concentrations effectively counteracted these adverse effects of glyphosate on biomass production and stem elongation. As shown in Table 1, the medium foliar Ni dose, corresponding to 0.42 mM Ni, was able to improve the biomass and, even more so, the height of plants sprayed with 1% of the recommended herbicidal dose of glyphosate, corresponding to 0.21 mM glyphosate. This relative concentration of foliar Ni to glyphosate is, in theory, more than sufficient to make an interaction between Ni and glyphosate in a 1:1 ratio possible. As discussed below, such an interaction could take part in the alleviation of the toxic effects of glyphosate by prior foliar Ni treatment. However, once the glyphosate level was increased from 1% (0.21 mM) to just 1.5% (0.32 mM), neither the medium (0.42 mM) nor the high (0.84 mM) foliar Ni dose provided any benefit at all (Figure 1; Table 1). These intriguing results suggest that the protective role of Ni against glyphosate drift damage may involve more complex mechanisms than a direct interaction.

Accumulation of shikimic acid due to inhibition of EPSPS is well documented in glyphosate-treated sensitive plants, particularly in the youngest tissues.[5,7] In this study, despite the devastating effects of both 1 and 1.5% glyphosate on plant growth parameters, significant shikimate accumulation could be detected only in 1.5% glyphosate-treated plants (Table 1C). The youngest leaves at the time of glyphosate application turned completely necrotic in 2 weeks in the 1.5% glyphosate treatment group, and they could therefore not be analyzed for shikimate accumulation at harvest time. Possibly, if they had been analyzed instead of the second youngest leaves at an earlier stage, significant shikimate accumulation could have been detected also in the 1% glyphosate group, and also dramatically higher shikimate concentrations could have been measured in the 1.5% glyphosate group.

The economic burden of glyphosate drift to nontarget crops is mainly due to losses in yield. Some glyphosate-sensitive crops such as soybean and cotton may partially or fully recover from glyphosate drift injury at early developmental stages,[8,9] whereas

Table 4. Germination Percentage and Shoot (Coleoptile + Primary Leaf) Length of 8-Day-Old Durum Wheat (*Triticum durum* cv. Balcali2000) Seedlings Grown in Perlite from Seeds Produced by Plants Treated with Foliar Ni and Glyphosate at Booting

foliar Ni (%)	glyphosate dose (% of rec)	germination percentage[a] (%)	shoot length[b] (cm)
0	0	86 ± 5	8.8 ± 1.0
	0.5	79 ± 7	8.6 ± 0.3
	1.0	69 ± 7	6.1 ± 1.1
0.01	0	89 ± 10	8.7 ± 0.6
	0.5	87 ± 7	8.7 ± 0.6
	1.0	81 ± 8	8.2 ± 0.3

[a]Germination percentage: $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (10; 7; ns). [b]Shoot length: $HSD_{0.05}$ (Gly; F.Ni; GlyxF.Ni) = (1.1; 0.7; 1.9).

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the grain yield of cereal crops such as wheat, corn, and rice is much more sensitive to sublethal glyphosate.[17,18,20,21] When compared to vegetative parts, generative tissues of plants are known to be more sensitive to glyphosate injury as they can accumulate much higher levels of glyphosate by acting as terminal sinks and are probably even more dependent on the products of the shikimic acid pathway.[7,43] Another reason for the higher sensitivity of generative tissues to glyphosate might be the relatively low concentration of some divalent nutrient cations with limited phloem mobility such as Ca, Mn, and Fe.[7] Possibly, reduced in planta complexation of glyphosate with these metals might potentiate glyphosate damage in reproductive organs.

According to Deeds et al.[13] and Roider et al.,[14] wheat yield is most sensitive to glyphosate when the generative organ primordia are developing. In practice, glyphosate is commonly applied as a preplant burn-down to summer crop fields in the early spring when wheat plants in nearby fields may be particularly susceptible to glyphosate drift injury. Depending on the glyphosate rate and the application stage, yield losses between 20 and 85% were observed in this study, and these losses could be to a significant extent prevented by foliar Ni treatment (Tables 2 and 3). It is noteworthy that the glyphosate rate (1% of the recommended concentration) responsible for dramatic yield losses in this greenhouse experiment was close to the lowest rate used in glyphosate drift simulation studies conducted under field conditions where drift doses as high as 12.5% were tested.[14−16] Although glyphosate can be slowly degraded to less toxic compounds in at least some species such as soybean, dilution due to continuing biomass production is the major way of gradual detoxification of sublethal glyphosate in planta.[2,18] Here, wheat subjected to simulated glyphosate drift at booting suffered greater yield reductions than wheat treated with the same glyphosate rate at tillering (Table 3), possibly because earlier applied glyphosate was diluted when plants reached the most glyphosate sensitive stage of their reproductive development.

The marked correlation between the grain yield and the number of grains produced per plant indicates that glyphosate reduces the yield by disrupting the grain formation rather than the grain filling (Figure 5A). Notably, the effect of glyphosate on the grain yield correlates also quite well with its effect on the final plant height (Figure 5B), in agreement with Deeds et al.[13] reporting that visual glyphosate injury is a reliable indicator for yield loss in wheat. However, glyphosate treatments had no effect on the straw dry weight (Tables 2 and 3). These findings conform to the literature indicating higher susceptibility of generative organs to glyphosate injury than vegetative biomass production.[7,43]

The negative effects of glyphosate on shoot dry weight of young plants, stem elongation, and grain yield of wheat were alleviated or eliminated by foliar Ni applications at sufficiently high rates (Tables 1−3), but not by soil Ni fertilization (Tables 1 and 2). This situation can be explained by the shoot and grain Ni concentrations, which did not respond to soil Ni application under the present experimental conditions but significantly increased with foliar Ni. The calcareous nature of the soil (described above) might have restricted the bioavailability of soil-applied Ni. Moreover, this finding may be explained by limited shoot translocation of soil Ni. Nickel is known to be mobile and translocated to growing parts of wheat,[44] but physiologically excess amounts of Ni taken up by the roots are sequestered in the root system and not translocated to the shoot of wheat, which is known as a Ni excluder.[45] In the case of foliar Ni application, leaf apoplastic Ni could account for part of the shoot and straw Ni concentrations reported in Tables 1 and 2, although the samples were washed thoroughly after harvest. However, the grain Ni concentration results provide evidence for the uptake and retranslocation of foliar-applied Ni. Remarkably, neither foliar Ni nor glyphosate applications caused any reduction in the grain concentrations of Ca, Mg, Zn, and Fe (data not shown).

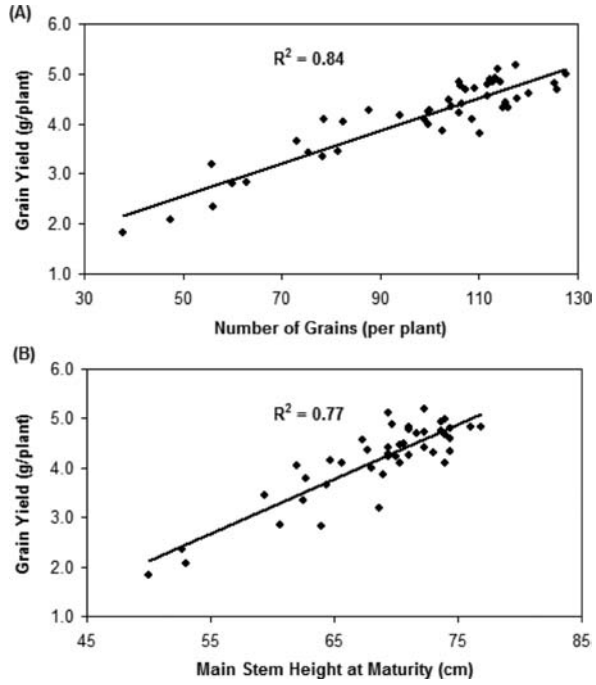

Figure 5. Correlation between (A) grain yield and number of grains produced per plant and (B) grain yield and main stem height at maturity for durum wheat (*T. durum* cv. Balcali2000) plants grown for the glyphosate drift simulation study under greenhouse conditions. (Data points are taken from Table 3.)

Despite the facts that Ni is an essential micronutrient for all higher plants[32,33] and its deficiency can be observed even under field conditions,[36] the positive effects of foliar Ni applications in the present study cannot be explained by the correction of Ni deficiency; because under given conditions, Ni applications did not provide any benefit in the absence of glyphosate treatment, and glyphosate treatments did not lower the Ni concentrations in any plant part analyzed (Tables 1−3). It is well documented that sublethal doses of glyphosate can interfere specifically with the uptake and/or translocation of Ca, Mg, Fe, and Mn in nontarget plants.[7,10,23,25] The possibility of an in planta interaction between glyphosate and Ni was investigated by Zobiole et al.,[31] who reported reduced leaf Ni concentrations in GR soybean upon glyphosate application and suggested that restricted Ni availability to symbiotic bacteria could be responsible for impaired N_2 fixation in glyphosate-treated soybean. However, according to the results presented here, drift doses of glyphosate do not affect Ni levels in wheat (Tables 1−3).

It is a well documented phenomenon that tank-mixing of divalent nutrients such as Mn, Zn, Ca, and Fe with glyphosate or even hard water can reduce the phytotoxicity of glyphosate, most probably because of the formation of glyphosate−metal complexes. This complex formation may affect the cuticular

penetration[28,30] and/or cellular uptake of glyphosate by diffusion or active transport mechanisms possibly involving phosphate transporters.[46] Losses in glyphosate efficacy due to foliar nutrient applications can be avoided by applying them separately, preferably later than glyphosate.[27] This tank-mix effect can also be eliminated in most cases when foliar nutrients are applied prior to glyphosate.[29] Because in the present study the purpose of foliar Ni applications was not just Ni fertilization but the protection of nontarget plants from possible glyphosate drift damage, Ni was applied separately to wheat plants a few days before glyphosate and not tank-mixed with it. Nevertheless, Ni ions remaining on the cuticle and in the apoplast may have interacted with glyphosate, interfered with its uptake, and inactivated it.

The protective effect of Ni against glyphosate drift in wheat may also be based on the inhibitory role of Ni in ethylene biosynthesis.[33,38,41] It was documented that plants subjected to sublethal glyphosate can produce higher levels of ethylene.[21,22] Application of ethephon, which is converted into ethylene in plants, to grasses at vegetative growth causes anomalies remarkably similar to glyphosate symptoms, including excessive tillering and height reduction.[47,48] Elevated levels of ethylene in the ambient air as well as ethephon applications can reduce wheat yield by inducing male sterility,[49,50] which may also be the cause of disrupted grain setting in glyphosate-affected wheat (Figure 5A; Table 3). However, further studies are required to clarify if glyphosate injury is linked to ethylene and also if inhibition of ethylene synthesis by Ni is behind its protective role against glyphosate.

The results presented herein show that glyphosate drift affects not only the wheat yield (Tables 2 and 3) but also the physical quality (Figure 4A) and germination capacity of wheat grain (Figure 4B; Table 4), in contrast to the results by Deeds et al.,[13] who claimed that glyphosate did not impair the germination of harvestable wheat grains. Apparently, foliar Ni applications can prevent the detrimental effects of glyphosate on the germination capacity of harvestable seeds as well as the seedling vigor (Figure 4B; Table 4), which might have severe implications on the yield of the next generation.

The most commonly used herbicide, glyphosate is still gaining popularity with the increasing adoption of glyphosate-resistant crops and no-tillage cropping systems. Glyphosate drift to nontarget crops in nearby fields is a growing practical problem and can cause serious economic losses, mainly due to its detrimental effects on yield. Wheat, which is a very important staple crop, is highly susceptible to glyphosate injury, particularly at early stages of generative development. The results presented in this study indicate that glyphosate rates as low as 1% or even 0.5% of the recommended herbicidal rate can disrupt seed set and thus significantly reduce wheat yield under controlled conditions. Foliar Ni applications at sufficiently high concentrations can apparently enhance the resistance of wheat to glyphosate drift damage. Not only yield loss but also the adverse effects of glyphosate on plant growth and seed quality can be partially or totally prevented by foliar Ni treatment. Direct binding of Ni to glyphosate and/or the role of Ni as an ethylene inhibitor may be behind the reported protective effects of Ni, but the exact mechanism remains to be elucidated. Foliar Ni application appears to have a great potential as a means to eliminate glyphosate drift injury to wheat and possibly other nontarget crops and should be optimized under field conditions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supporting Table 1: Shoot dry weight, main stem height and shoot Ni concentration of 43-day-old durum wheat (*Triticum durum* cv. Balcali2000) plants treated with foliar Ni (27 DAS) and glyphosate (29 DAS) at tillering under greenhouse conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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